## WHAT IS CLAIMED IS:

1. A compound having the formula:

wherein B is a nucleobase; L is a linker;  $R_3$  is triphosphate,  $\alpha$ -thiotriphosphate, or a salt thereof, and Dye is a reporter group

- 2. The compound according to claim 1, wherein the linker is an propargyl-ethyloxide-amino linker.
- The compound according to claim 1, wherein the linker is a propargyl linker.
- 4. The compound according to claim 1, wherein the linker is a benzylamine linker.
- 5. The compound according to claim 1, wherein the linker is a phosphate linker.
- The compound according to claim 1, wherein the linker is a rigid linker.
- 7. The compound according to claim 1, wherein the linker is a tuned linker.
- 8. The compound according to claim 1, wherein the linker is a heterocycle linker.

- 9. The compound according to claim 1, wherein the dye is a fluorescein-type dye, a rhodamine type dye, an energy transfer dye pair, or a cyanine-type dye.
- 10. The compound according to claim 9, wherein Dye is selected from a rhodamine dye and a fluorescein dye.
- 11. The compound according to claim 10, wherein the rhodamine dye is a 4,7-dichlorophenyl-rhodamine dye.
- 12. The compound according to claim 11, wherein the 4,7-dichlorophenyl-rhodamine dye is selected from dTAMRA, dROX, dR6G, and dR110.
- 13. The compound according to claim 1, wherein the nucleobase is selected from cytosine, adenine, uracil, guanine, 7-deazaadenine, and 7-deazaguanine.

## 14. A compound of the formula I:

Dye 
$$(L_2)_n$$
 $(L_1)_m$ 
 $(L_1)_m$ 
 $(L_2)_n$ 
 $(L_2)_n$ 
 $(L_2)_n$ 
 $(L_3)_n$ 
 $(L_4)_m$ 
 $(L_2)_n$ 
 $(L_2)_n$ 
 $(L_4)_m$ 
 $(L_4)_m$ 

- wherein X is N, NH, or C;
- wherein Y is O or  $NH_2$ ;
- wherein  $R_3$  is either triphosphate,  $\alpha$ -thiotriphosphate, or a salt thereof;
- wherein L<sub>1</sub> is a linker;
- wherein L<sub>2</sub> is a a benzylamine linker or a phosphate linker;
- wherein n = 0-4, m = 0-4, and m + n is at least 1; and;
- wherein the dye is any reporter group.
- 15. The compound according to claim 14, wherein X is N and Y is NH<sub>2</sub>.
- 16. The compound according to claim 14, wherein X is C and Y is O.
- 17. The compound according to claim 14 wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker, a propargylamino linker, a propargyl-propyl-oxide-amino linker, a benzylamine linker, a phosphate linker, a rigid linker, or a multimer thereof.

- 18. The compound according to claim 14, wherein m = 1 and  $L_2$  is a benzylamine linker.
- 19. The compound according to claim 18, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker or a propargylamino linker.
- 20. The compound according to claim 14, wherein m=1 and  $L_2$  is a phosphate linker.
- 21. The compound according to claim 20, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker or a propargylamino linker.
- 22. A compound of the formula II:

- wherein L is a linker;
- wherein R<sub>4</sub> is either NH<sub>2</sub>, OH, or O, and B is either NH<sub>2</sub>, OH, or H;
- wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof; and
- wherein the dye is any reporter group.

The first force from the first first

- 23. The compound according to claim 22, wherein the linker is a propargyl-ethyl-oxide-amino linker, a propargylamino linker, a propargyl-propyl-oxide-amino linker, a benzylamine linker, a phosphate linker, a rigid linker, or a multimer thereof.
- 24. The compound according claim 22, wherein the linker is an propargyl-ethyloxide-amino linker.
- 25. The compound according to claim 22, wherein the linker is a propargylamino linker.
- 26. A compound of the formula III:

Dye 
$$(L_2)$$
n  $(L_1)$ n  $R_4$  Formula III  $R_3$   $R_5$ 

- wherein L<sub>1</sub> is a linker;
- wherein L<sub>2</sub> is a a benzylamine linker or a phosphate linker;
- wherein n = 0-4, m = 0-4, and m + n is at least 1;
- wherein  $R_4$  is either  $NH_2$ , OH, or O, and  $R_5$  is either  $NH_2$ , OH, or H;
- wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof; and
- wherein the dye is any reporter group.

Attorney Docket No.: 07414.0040

- 27. The compound according to claim 26, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker, a propargylamino linker, a propargyl-propyl-oxide-amino linker, a benzylamine linker, a phosphate linker, a rigid linker, or a multimer thereof.
- 28. The compound according to claim 26, wherein n = 1 and  $L_2$  is a benzylamine linker.
- 29. The compound according to claim 28, wherein  $L_1$  is a propargylethyl-oxide-amino linker or a propargylamino linker.
- 30. The compound according to claim 26, wherein n = 1 and  $L_2$  is a phosphate linker.
- 31. The compound according to claim 30, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker or a propargylamino linker.
- 32. The compound according to claim 28, wherein R<sub>4</sub> is NH<sub>2</sub> and R<sub>5</sub> is H.
- 33. The compound according to claim 28, wherein  $R_4$  is O and  $R_5$  is  $NH_2$ .

34. A compound of the formula IV:

$$R_{3}$$
 $R_{4}$ 
 $R_{7}$ 
 $R_{1}$ 
 $R_{1}$ 
 $R_{2}$ 
Formula IV

- wherein R<sub>1</sub>, R<sub>2</sub>, and R<sub>4</sub> are independently H, O, OR, S, SR, NR<sub>2</sub> or CR<sub>2</sub>;
- wherein R<sub>3</sub> is SR, NR<sub>2</sub>, OR, or CR<sub>2</sub> and comprises a reporter group;
- wherein R is hydrogen, alkyl, aryl, or an amino acid;
- wherein R<sub>7</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein X, Y, and Z are independently carbon, nitrogen, oxygen, sulfur, phosphorus, or selenium;
  - wherein n is 0 or 1; and
  - wherein M is H<sub>2</sub>O or any metal.
- 35. The compound according to claim 34, wherein R is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, benzyl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>, or an amino acid and further wherein R' is H, OH, or alkyl.
- 36. The compound according to claim 34, wherein n = 0.

- 37. The compound according to claim 34, wherein n = 1 and further wherein M is a Group IA metal or a Group IIA metal
- 38. The compound according to claim 37, wherein M is Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, or Ca<sup>2+</sup>.
- 39. The compound according to claim 34, wherein R<sub>1</sub> is NH<sub>2</sub> and R<sub>2</sub> is H.
- 40. The compound according to claim 34, wherein  $R_1$  is O and  $R_2$  is  $NH_2$ .
- 41. The compound according to claim 35, wherein R' is H, OH, C1-C6 alkyl, or C2-C6 alkynyl.
- 42. A compound of the formula V:

$$R_{7}$$
 $N$ 
 $R_{1}$ 
 $R_{2}$ 
Formula V
 $R_{7}$ 
 $R_{1}$ 
 $R_{2}$ 

- wherein R<sub>1</sub>, R<sub>2</sub>, and R<sub>4</sub> are independently H, O, OR, S, SR, NR<sub>2</sub> or CR<sub>2</sub>;
- wherein R<sub>3</sub> is SR, NR<sub>2</sub>, OR, or CR<sub>2</sub> and comprises a reporter group;
- wherein R is hydrogen, alkyl, aryl, or an amino acid;

- wherein R<sub>7</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein X, Y, and Z are independently carbon, nitrogen, oxygen, sulfur, phosphorus, or selenium;
  - wherein n is 0 or 1; and
  - wherein M is H<sub>2</sub>O or any metal.
- 43. The compound according to claim 42, wherein R is hydrogen, C1-C6 alkyl, C2-C6 alkynyl, C2-C6 alkenyl, benzyl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>, or an amino acid and further wherein R' is H, OH, or alkyl.
- 44. The compound according to claim 42, wherein n = 0.
- 45. The compound according to claim 42, wherein n = 1 and further wherein M is a Group IA metal or a Group IIA metal
- 46. The compound according to claim 45, wherein M is Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, or Ca<sup>2+</sup>.
- 47. The compound according to claim 42, wherein  $R_1$  is  $NH_2$  and  $R_2$  is H.
- 48. The compound according to claim 42, wherein  $R_1$  is O and  $R_2$  is  $NH_2$ .

- 49. The compound according to claim 43, wherein R' is H, OH, C1-C6 alkyl, or C2-C6 alkynyl.
- 50. A compound of the formula VI:

$$R_{5}$$
 $R_{1}$ 
 $R_{1}$ 
 $R_{2}$ 
 $R_{1}$ 
 $R_{2}$ 
 $R_{3}$ 
 $R_{4}$ 
 $R_{5}$ 
 $R_{5$ 

- wherein R<sub>1</sub> is H, O, OR, S, SR, NR<sub>2</sub>, or CR<sub>2</sub>,
- wherein R<sub>2</sub> is SR, NR<sub>2</sub>, OR, or CR<sub>2</sub> and comprises a reporter group;
- wherein R is hydrogen, alkyl, alkynyl, aryl, or an amino acid;
- wherein R<sub>5</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein X is N, NH, or C;
- wherein Y is O or NH<sub>2</sub>;
- wherein A, B, and E are independently C, N, O, S, P, or Se;
- wherein n is 0 or 1; and
- wherein M is  $H_2O$  or any metal.
- 51. The compound according to claim 50, wherein X is N and Y is NH<sub>2</sub>.

- 52. The compound according to claim 50, wherein X is C and Y is O.
- The compound according to claim 50, wherein R is hydrogen, C1-C6 alkyl, C2-C6 alkynyl, C2-C6 alkenyl, benzyl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>, or an amino acid and further wherein R' is H, OH, or alkyl;
- 54. The compound according to claim 50, wherein n = 0.
- 55. The compound according to claim 50, wherein n = 1 and further wherein M is a Group IA metal or a Group IIA metal
- 56. The compound according to claim 50, wherein M is Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, or Ca<sup>2+</sup>.
- 57. The compound according to claim 53, wherein R' is H, OH, C1-C6 alkyl, or C2-C6 alkynyl.
- 58. A compound of the formula VII:

- wherein A is NH<sub>2</sub>, OH, or O;

- wherein R is H, O, NR'2, S, CR'2, or halide;
- wherein R' is hydrogen or alkyl;
- wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein L is alkyl;
- wherein X is CR or N and Y is O, S, or NH; and
- wherein the dye is any reporter group.
- 59. The compound according to claim 58, wherein R' is C1-C20 alkyl, C2-C20 alkenyl, or C2-C20 alkynyl, or aryl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>.
- 60. The compound according to claim 58, wherein R is fluorine, bromine, iodine, or chlorine.
- 61. The compound according to claim 58, wherein R is fluorine or chlorine.
- 62. The compound according to claim 58, wherein R' is hydrogen or C1-C7 alkyl.

## 63. A compound of the formula VIII:

- wherein X is N, NH, or C;
- wherein Y is O or NH<sub>2</sub>;
- wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein A is O, S, or NH;
- wherein L is alkyl or aryl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>;
  - wherein R' is hydrogen or alkyl;
  - wherein n is 1 to 10; and
- wherein the dye is any reporter group, preferably a rhodamine-type dye, a fluorescein-type dye, an energy transfer dye, or a cyanine-type dye.
- 64. The compound according to claim 63, wherein L is C1-C20 alkyl, C2-C20 alkenyl, C2-C20 alkynyl, or benzyl substituted at from 0 to 3 positions in a chemically reasonable

manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>.

- 65. The compound according to claim 63, wherein R' is hydrogen or C1-C7 alkyl.
- 66. The compound according to claim 63, wherein X is N and Y is NH<sub>2</sub>.
- 67. The compound according to claim 63, wherein X is C and Y is O.
- 68. A compound of the formula IX:

Dye 
$$R$$
  $H$   $N$   $R_4$   $R_5$  Formula IX  $R_3$   $R_5$ 

- wherein  $R_4$  is  $NH_2$ , OH, or O and  $R_5$  is  $NH_2$ , OH, or H, provided that if A is  $NH_2$ , B is H and if A is O, B is  $NH_2$ ;
  - wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
  - wherein the dye is any reporter group; and
  - wherein R is a side chain for mobility tuning.
- 69. The compound according to claim 68, wherein R is a hydrophilic side chain.

- 70. The compound according to claim 68, wherein R is a hydrophobic side chain.
- 71. The compound according to claim 68, wherein  $R_4$  is  $NH_2$  and  $R_5$  is H.
- 72. The compound according to claim 68, wherein  $R_4$  is O and  $R_5$  is  $NH_2$ .
- 73. A compound of the formula X:

- wherein X is N, NH, or C;
- wherein Y is O or NH<sub>2</sub>;
- wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein Dye is any reporter group, and
- wherein R is a side chain for mobility tuning.
- 74. The compound according to claim 73, wherein R is a hydrophilic side chain.

- 75. The compound according to claim 73, wherein R is a hydrophobic side chain.
- 76. The compound according to claim 73, wherein X is N and Y is NH<sub>2</sub>.
- 77. The compound according to claim 73, wherein X is C and Y is O.
- 78. A compound having the formula:

- wherein B is a nucleobase selected from uracil, cytosine, adenine, 7-deazaguanine;
  - wherein R<sub>3</sub> is triphosphate or a salt thereof;
- wherein L is a linker selected from propargyl-ethyl-oxide-amino and propargylamino wherein the linker is attached to the 8-C of a adenine, 7-deazaadenine, guanine, or 7-deazaguanine nucleobase, the 7-C or 8-C of a 7-deazaadenine or 7-deazaguanine nucleobase, or the C-5 of a uracil or cytosine nucleobase; and
  - wherein Dye is selected from a rhodamine dye and a fluorescein dye.
- 79. The compound according to claim 78, wherein B is uracil; L is propargyl-ethyloxide-amino; and Dye is TAMRA.

- 80. The compound according to claim 78, wherein B is uracil; L is propargylamino; and Dye is TAMRA.
- 81. The compound according to claim 78, wherein B is uracil; L is propargyl-ethyloxide-amino; and Dye is dTAMRA.
- 82. The compound according to claim 78, wherein B is uracil; L is propargylamino; and Dye is dTAMRA.
- 83. The compound according to claim 78, wherein B is cytosine; L is propargyl-ethyloxide-amino; and Dye is ROX.
- 84. The compound according to claim 78, wherein B is cytosine; L is propargylamino; and Dye is ROX.
- 85. The compound according to claim 78, wherein B is cytosine; L is propargyl-ethyloxide-amino; and Dye is dROX.
- 86. The compound according to claim 78, wherein B is cytosine; L is propargylamino; and Dye is dROX.
- 87. The compound according to claim 78, wherein B is 7-deazaadenine; L is propargyl-ethyl-oxide-amino; and Dye is R6G.

Attorney Docket No.: 07414.0040

- 88. The compound according to claim 78, wherein B is 7-deazaadenine; L is propargylamino; and Dye is R6G.
- 89. The compound according to claim 78, wherein B is 7-deazaadenine; L is propargyl-ethyl-oxide-amino; and Dye is dR6G.
- 90. The compound according to claim 78, wherein B is 7-deazaadenine; L is propargylamino; and Dye is dR6G.
- 91. The compound according to claim 78, wherein B is 7-deazaguanine; L is propargyl-ethyl-oxide-amino; and Dye is R110.
- 92. The compound according to claim 78, wherein B is 7-deazaguanine; L is propargylamino; and Dye is R110.
- 93. The compound according to claim 78, wherein B is 7-deazaguanine; L is propargyl-ethyl-oxide-amino; and Dye is dR110.
- 94. The compound according to claim 78, wherein B is 7-deazaguanine; L is propargylamino; and Dye is dR110.
- 95. The compound according to claim 78, wherein Dye is an energy transfer dye pair comprised of a fluorescein dye and a rhodamine dye wherein the fluorescein dye is

nt i

attached to the nucleobase through a propargyl-ethyl-oxide-amino or propargylamino linker, and the fluorescein dye is attached to the rhodamine dye by a second linker.

- 96. The compound according to claim 95, wherein the fluorescein dye is 4-aminomethyl FAM and the rhodamine dye is a 4,7-dichlororhodamine dye.
- 97. The compound according to claim 96, wherein the 4,7-dichlororhodamine dye is dTAMRA.
- 98. The compound according to claim 96, wherein the 4,7-dichlororhodamine dye is dROX.
- 99. The compound according to claim 96, wherein the 4,7-dichlororhodamine dye is dR6G.
- 100. The compound according to claim 96, wherein the 4,7-dichlororhodamine dye is dR110.
- 101. A method for determining the sequence of a DNA template, comprising
  - (i) annealing at least one oligonucleotide primer to a template;
- (ii) incubating said at least one oligonucleotide primer with a DNA polymerase that can incorporate both dNTPs and rNTPs in a reaction comprising a mixture of

unlabeled dNTPs and at least one dye-labeled ribonucleotide of the invention so that primer extension products are formed;

- (iii) treating the primer extension products with a means for hydrolyzing the extension products at each occurrence of a ribonucleotide;
- (iv) separating the resulting fragments that contain said at least one primer from other fragments,
  - (v) resolving the primer-containing extension products by size; and
  - (vi) detecting the fragments.
- 102. The method according to claim 101, wherein the dye-labeled ribonucleotides are rATP-PA-6R6G, rCTP-PA-Rox, rUTP-PA-Tamra and rGTP-EO-R110.
- 103. The method according to claim 101, wherein one primer is biotinylated.
- 104. The method according to claim 101, wherein at least one primer is a hybridization based pull-out primer.
- 105. The method according to claim 101, wherein the DNA polymerase is a thermostable DNA polymerase.
- 106. The method according to claim 105, wherein the thermostable DNA polymerase is a modified thermostable DNA polymerase having increased efficiency for the incorporation of ribonucleotides.

- 107. The method according to claim 101, wherein the means for hydrolyzing the extension products at each occurrence of a ribonucleotide is alkali treatment, heat treatment, or a ribonuclease.
- 108. A method for detecting mutations in DNA, comprising
  - annealing two oligonucleotide primers to a template;
- incubating the two oligonucleotide primers with a DNA polymerase that can incorporate both dNTPs and rNTPs in a reaction comprising a mixture of unlabeled dNTPs and at least one dye-labeled ribonucleotide of the invention so that primer extension products are formed;
- treating the primer extension products with a means for hydrolyzing the extension products at each occurrence of a ribonucleotide to produce fragments;
  - resolving the fragments by size; and
  - detecting the fragments.
- 109. The method according to claim 108, wherein the fragments that contain primers are separated from other fragments before the fragments that contain primers are resolved by size.
- 110. The method according to claim 108, wherein the mutation is a single nucleotide polymorphism.
- 111. The method according to claim 108, wherein the DNA is genomic DNA.

- 112. The method according to claim 108, wherein at least one primer is biotinylated.
- 113. The method according to claim 108, wherein at least one primer is a hybridization based primer.
- 114. The method according to claim 108, wherein one primer comprises a modified base preventing primer extension in the 5' direction.
- 115. A method for preparing polynucleotide fragments, comprising
- incubating a DNA template with a DNA polymerase, dATP, dGTP, dCTP, dTTP, at least two oligonucleotide primers complementary to the DNA template, and at least one dye-labeled ribonucleotide so that the primers are extended and said at least one dye-labeled ribonucleotide is incorporated in the primer extension products; and
- hydrolyzing 3'-5' phosphodiester linkages between adjacent ribo- and deoxyribonucleotides.
- 116. The method according to claim 115, wherein the 3'-5' phosphodiester linkages between adjacent ribo- and deoxyribonucleotides are hydrolyzed by alkali, heat, or a ribonuclease.
- 117. A method for preparing dye-labeled RNA complementary to a sequence of interest comprising:

- preparing a mixture of a template, an RNA polymerase, rATP, rGTP, rCTP, rUTP, and at least one dye-labeled ribonucleotide, wherein said sequence of interest is operably linked to a site for the initiation of RNA synthesis by the RNA polymerase; and

- incubating the mixture so that the RNA polymerase catalyzes the synthesis of RNA.

- 118. A method for detecting 5-methylcytosine in a DNA template comprising:
- treating the DNA template with a bisulfite salt under conditions whereby 5methylcytosine remains non-reactive;
- incubating the DNA template with a DNA polymerase, dATP, dGTP, dCTP, dTTP, at least two oligonucleotide primers complementary to the DNA template, and a dye-labeled rCTP compound so that the primers are extended and the dye-labeled rCTP compound is incorporated in the primer extension products;
- hydrolyzing 3'-5' phosphodiester linkages between adjacent ribo- and deoxyribonucleotides to produce fragments;
  - resolving the fragments by size; and
  - detecting the fragments.
- 119. The method according to claim 118, wherein the template DNA is genomic DNA.
- 120. The method according to claim 118, wherein the fragments that contain primers are separated from other fragments before the fragments that contain primers are resolved by size.

Attorney Docket No.: 07414.0040

- 121. The method according to claim 118, wherein at least one primer is biotinylated.
- 122. The method according to claim 118, wherein at least one primer is a hybridization based primer.
- 123. The method according to claim 118, wherein one primer comprises a modified base preventing primer extension in the 5' direction.